

IN THE SPECIFICATION:

At page 1, line 5, please add the following paragraph:

GOVERNMENTAL SUPPORT

A1 The research leading to the present invention was supported, at least in part, by a grant from the National Institutes of Health, Grant No. CA81102 and Grant No. GM57300. Accordingly, the Government may have certain rights in the invention.

Please replace the paragraph beginning at page 1, lines 8 through 11 with the following paragraph.

A2 The present application is a continuation-in-part of copending application Serial No. 09/632,131 filed August 3, 2000, of which the instant application claims the benefit of the filing date pursuant to 35 U.S.C. § 120, and which is incorporated herein by reference in its entirety. The present application claims priority pursuant to 35 U.S.C. § 119(e) to provisional application Serial No. 60/188,957 filed March 13, 2000, which is incorporated herein by reference in its entirety.

Please replace the paragraph beginning at page 3, line 12 and continuing to page 4, line 26.

A3 Evidence for MHCK and eEF-2 kinase forming the core of a new superfamily is as follows. MHCK A from *Dictyostelium*, has a demonstrated role in the regulation of myosin assembly (Futey et al., (1995) *J. Biol. Chem.* 270:523-529; Côté et al., (1997) *J. Biol. Chem.* 272:6846-6849). eEF-2 kinase is a ubiquitous  $\text{Ca}^{2+}$ /calmodulin-dependant protein kinase involved in the regulation of protein synthesis by  $\text{Ca}^{2+}$  (Redpath et al., (1996) *J. Biol. Chem.* 271:17547-17554; Ryazanov et al., (1997) *Proc. Natl. Acad. Sci., USA* 94:4884-4889). Both MHCK A and eEF-2 kinase display no homology to any of the known protein kinases, but are strikingly similar to each other; amino acid sequences of their catalytic domains are 40% identical. Another protein

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kinase homologous to MHCK A and eEF-2 kinase has recently been identified in *Dictyostelium* (Clancy et al., (1997) *J. Biol. Chem.* 272:11812-11815), and an expressed sequence tag (EST) sequence, with a high degree of similarity to the catalytic domain common to both MHCK A and eEF-2 kinase, has been deposited in GenBank (clone FC-AN09/accession #C22986). An amino acid sequence alignment of the catalytic domains of these new protein kinases is shown in Figure 1A. These kinases have a catalytic domain of approximately 200 amino acids which can be subdivided into seven conserved subdomains. Subdomains V, VI, and VII have a predicted  $\beta$ -sheet structure and are presumably involved in ATP-binding, while subdomains I through IV may be involved in substrate binding and catalysis. These new protein kinases have no homology to the members of the eukaryotic serine/threonine/tyrosine protein kinase superfamily with the exception of the GXGXXG (SEQ ID NO:21) motif in subdomain VI which is present in many ATP-binding proteins. Thus, MHCK A, eEF-2 kinase, and related protein kinases may represent a new superfamily. Evolutionary analysis of these new kinases (Fig. 1B) reveals that they can be subdivided into 2 families: the eEF-2 kinase family which includes eEF-2 kinases from different organisms, and the MHCK family which includes MHCK A, MHCK B and FC-AN09. These two families appear to have split more than a billion years ago.

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Please replace the paragraph at page 20, line 19 through line 25.

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**FIGURE 1 A and B.** A, Sequence alignment of the catalytic domains of human eEF-2 kinase, *C. elegans* eEF-2 kinase, MHCK A, MHCK B and clone FC-ANO9. Identical amino acids (bold) and conserved hydrophobic amino acids (<sup>o</sup>) are noted. B, Phylogenetic tree of sequences shown in (A), with the addition of mouse and rat eEF-2 kinases. Tree was obtained using the J. Hein method with PAM250 residue weight table. The following accession numbers were used for the sequences: U93846-U93850, 1495779, 1170675, 1903458, C22986.

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Please replace the paragraph at page 21, line 7 through line 10.

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**FIGURE 3** depicts a schematic representation of the structure of mammalian and *C. elegans*

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A5 eEF-2 kinases and MHCK A. The homologous regions are represented by dark shading. The regions of weak similarity are represented by light shading. The position of the GXGXXG (SEQ ID NO: 21) motif is indicated by vertical arrows.

Please replace the paragraph at page 21, line 18 through line 19.

AL6 **FIGURE 5 A through C** depicts the nucleic acid sequence of mouse melanoma alpha-kinase (MK).

Please replace the paragraph at page 21, line 21 through line 22.

A7 **FIGURE 6 A and B** depicts the predicted amino acid sequence of mouse melanoma alpha-kinase (MK).

Please replace the paragraph at page 22, line 18 through line 25.

A8 **FIGURE 15 A through F** shows Northern Blot analysis of the tissue distribution of the alpha-kinases in human and mouse tissues. Standard Multiple Tissue Northern (MTN) blots (Clontech) were stained as described in Materials and Methods. A, B, C: Blots probed for Melanoma Kinase; A: Human MTN Blot B: Human Immune System MTN Blot II. C: Mouse MTN Blot. D: Human 12-Lane MTN Blot probed for Kidney kinase. E: Human 12-Lane MTN Blot probed for Muscle kinase. F: Mouse MTN Blot probed for Heart kinase. (abbreviations: sk. muscle - skeletal muscle, p.b. leukocyte -peripheral blood leukocyte, s. intestine - small intestine).

Please replace the paragraph at page 23, line 10 through line 12.

A9 **FIGURE 19 A and B.** A. Phylogenetic tree of the LTRP channel subfamily. This tree was generated from the full-length protein sequences using the ClustalW program. B. The proposed structural model of MK and KK.

Complete Listing of Claims in Application U.S.S.N. 09/832,292

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Claims 1-3 (cancelled)

4. (Currently amended) An isolated nucleic acid encoding mammalian heart alpha kinase, wherein the nucleic acid is selected from the group consisting of :

- a. the DNA sequence of SEQ ID NO: 34;
- ~~b. the DNA sequence of SEQ ID NO: 36;~~
- b e. DNA sequences that hybridize to the sequence of subparts (a) ~~or (b)~~ under standard hybridization conditions; and
- c d. DNA sequences capable of encoding the amino acid sequence encoded by the DNA sequences of subparts (a) or ; (b) ~~or (c)~~.

Claim 5 (original)

Claims 6-13 (cancelled)

14. (Currently amended) A recombinant DNA expression vector comprising the nucleic acid of ~~any of~~ Claims ~~1, 4, 7, 10 or 12~~, wherein the DNA encoding the heart alpha kinase is operatively associated with an expression control sequence.

Claim 15 (original)

16. (Currently amended) A unicellular host transformed with a recombinant DNA molecule comprising a DNA sequence or degenerate variant thereof, which encodes an heart alpha kinase, or a fragment thereof, selected from the group consisting of:

- ~~a. the DNA sequence of (SEQ ID NO: 26);~~
- ~~b. the DNA sequence of (SEQ ID NO: 28);~~
- ~~c. the DNA sequence of (SEQ ID NO: 30);~~
- ~~d. the DNA sequence of (SEQ ID NO: 32);~~
- a e. the DNA sequence of (SEQ ID NO: 34);
- ~~f. the DNA sequence of (SEQ ID NO: 36);~~
- ~~g. the DNA sequence of (SEQ ID NO: 38);~~
- ~~h. the DNA sequence of (SEQ ID NO: 40);~~
- b i. DNA sequences that hybridize to ~~any of~~ the foregoing DNA sequences under standard hybridization conditions; and
- c j. DNA sequences that encode ~~code on expression for~~ an amino acid sequence encoded by any of the foregoing DNA sequences;  
wherein said DNA sequence is operatively linked to an expression control sequence.

Claim 17 (original)